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Changes in Soil Fungal Communities, Extracellular Enzyme Activities, and Litter Decomposition Across a Fire Chronosequence in Alaskan Boreal Forests

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Abstract

Wildfires are a pervasive disturbance in boreal forests, and the frequency and intensity of boreal wildfires is expected to increase with climate warming. Boreal forests store a large fraction of global soil organic carbon (C), but relatively few studies have documented how wildfires affect soil microbial communities and soil C dynamics. We used a fire chronosequence in upland boreal forests of interior Alaska with sites that were 1, 7, 12, 24, 55, ~90, and ~100 years post-fire to examine the short- and long-term responses of fungal community composition, fungal abundance, extracellular enzyme activity, and litter decomposition to wildfires. We hypothesized that post-fire changes in fungal abundance and community composition would constrain decomposition following fires. We found that wildfires altered the composition of soil fungal communities. The relative abundance of ascomycetes significantly increased following fire whereas basidiomycetes decreased. Post-fire decreases in basidiomycete fungi were likely attributable to declines in ectomycorrhizal fungi. Fungal hyphal lengths in the organic horizon significantly declined in response to wildfire, and they required at least 24 years to return to pre-fire levels. Post-fire reductions in fungal hyphal length were associated with decreased activities of hydrolytic extracellular enzymes. In support of our hypothesis, the decomposition rate of aspen and black spruce litter significantly increased as forests recovered from fire. Our results indicate that post-fire reductions in soil fungal abundance and activity likely inhibit litter decomposition following boreal wildfires. Slower rates of litter decay may lead to decreased heterotrophic respiration from soil following fires and contribute to a negative feedback to climate warming.

Key words: boreal forest; carbon cycle; chronosequence; climate change; decomposition; extracellular enzymes; fire; fungi; microbe.

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Introduction

Given the large amount of carbon (C) stored within boreal ecosystems, there is increasing interest in the role of boreal forests in the global C cycle and in feedbacks between climate change and the C cycle in these regions. Boreal ecosystems have already

warmed by ~1.5°C (Moritz and others 2002) and are anticipated to warm by an additional 4–7°C by the end of the century (ACIA 2004). One potential consequence of climate warming in boreal forests is an increase in the frequency and intensity of wildfires. Wildfires currently burn ~110,000 km^2 in boreal forests every year (Giglio and others 2006) and warmer and drier climate patterns, coupled with a longer growing season, provide increased opportunities for fire occurrence. In Alaskan boreal forests, wildfire occurrence has increased threefold in the past 30 years (Kasischke and Stocks 2000) and the 2000s experienced a larger burned area and number of extreme fire events than any other decade in the modern record period (Kasischke and others 2010). Modeling studies predict that the burned area in Alaskan and Canadian boreal forests will further increase 3.5–5.5 times by the end of the century (Balshi and others 2009). Boreal wildfires directly affect the C cycle via CO_2 emissions from biomass combustion, and indirectly through long-term changes in ecosystem C dynamics that occur during forest recovery and succession (Goulden and others 2011). Understanding how wildfires indirectly affect the C cycle, and particularly soil C dynamics, is critical for predicting feedbacks between climate change, wildfires, and the C cycle in boreal forests.

Soil microbial communities contribute to the transfer of C from terrestrial ecosystems to the atmosphere via the decomposition of organic matter (Swift and others 1979) and thus are likely to play a major role in mediating indirect wildfire feedbacks to the C cycle. Despite their role in mediating C cycle feedbacks, the response of decomposer organisms to boreal wildfires is uncertain. Previous studies have concluded that post-fire increases in soil temperature stimulate microbial activity (Burke and others 1997; Richter and others 2000; Kim and Tanaka 2003), leading to higher rates of organic matter decay and augmenting ecosystem C losses. However, these conclusions are primarily based on measurements of total soil respiration per unit area and are built on the assumption that the autotrophic component of soil respiration is negligible following fires (Richter and others 2000). Direct measurements of soil microbial activity or decomposition following wildfires are relatively scarce.

Putative increases in microbial activity following fires are difficult to reconcile with the observed response of soil microbial biomass to wildfires. Total microbial biomass, and fungal biomass specifically, generally declines following wildfires (Bååth and others 1995; Grady and Hart 2006; Dooley and Treseder 2012). Fires may negatively affect total soil microbial abundance through both direct and indirect mechanisms. Direct heat transfer to soils during fire may lead to heat-induced microbial mortality (Hart and others 2005; Cairney and Bastias 2007). In addition, fires alter soil physical and chemical properties like hydrophobicity, nutrient concentrations, and C quality, and these changes may in turn have negative consequences for microbes (Certini 2005). Five years following a wildfire in a boreal forest, Waldrop and Harden (2008) observed reductions in soil fungal biomass, oxidative enzyme activity, and lignin decomposition. These results suggest that decomposition rates may not increase following fires and instead that post-fire reductions in microbial abundance can reduce the potential of soil microbes to decompose soil C.

Soil microbial responses to fire are likely to change over the course of forest recovery and may only persist until aboveground plant communities regenerate (Hart and others 2005). Aboveground plant biomass can recover in as little as 4 years following boreal wildfires (Mack and others 2008). The rapid recovery of plant biomass following fire suggests that the response of microbial biomass to fire may also be ephemeral. In addition, compositional shifts in soil microbial communities in response to fire will likely change throughout forest secondary succession. Fire has previously been shown to stimulate the development of certain ascomycete fungi (Wicklow 1975). In contrast, mycorrhizal fungi may decline immediately following fires due to the absence of the appropriate host plants (Chen and Cairney 2002). Because soil microbial communities are influenced by plant community composition, microbial community composition likely shifts throughout forest secondary succession as the dominant plant species change. Overall, the timescale of microbial biomass and community composition changes following boreal wildfires is poorly resolved.

In this study, we characterized the short- and long-term response of soil fungal communities to boreal wildfires. We used a fire chronosequence in boreal forests of interior Alaska to test the hypothesis that post-fire changes in microbial abundance and community composition constrain decomposition in post-fire soils (sensu Waldrop and Harden 2008). We predicted that fires would reduce soil fungal abundance and alter fungal community composition. Furthermore, we expected that post-fire reductions in fungal abundance would decrease litter decay rates following wildfires. Finally, we predicted that fungal responses to fire would be transient and would persist until

aboveground plant biomass recovered. We chose to focus primarily on soil fungi because of their high abundance in boreal forest soils and because of their central role in the decomposition of recalcitrant C compounds in soil.

Materials and Methods

Study Sites

This study was conducted in the Donnelly Flats area located near Delta Junction in interior Alaska. We sampled from boreal forest stands that burned in severe fires during the summers of 2010, 2004, 1999, 1987, and 1956 (Appendix A in Supplementary Material). All fires were stand replacing and personal observations of white-ash deposits following the 2010 and 1999 fires indicate high burn temperatures at the soil surface. We also sampled from two control sites that were about 90 and about 100 years post-fire. Burned sites were separated from control sites by at least several hundred meters. Control sites were selected to have similar plant species composition and tree density to that of the burned sites and their ages were determined through tree coring and historical fire records. For simplicity, we refer to the time since fire for each site in 2011 even though some observations were made in 2010 and the figures reflect the actual time at observation. Sites were chosen to have similar climate, potential biota, topography, and parent material, to isolate the effects of fire on these ecosystems. Portions of this chronosequence have been used to assess the effects of fire on plant productivity (Mack and others 2008), soil CO_2 fluxes (O'Neill and others 2003), and mycorrhizal fungi (Treseder and others 2004).

Plant communities along the chronosequence represent several stages of secondary succession (Table 1). Soils are Inceptisols and the local climate is cold and dry with a mean annual precipitation rate of 303 mm y^{-1} and a mean annual temperature of 2°C (http://weather.noaa.gov/). The growing season extends from bud-break in mid-May to leaf senescence in mid-September. Permafrost is discontinuous in the region and is not present at these sites.

Soil Sampling

We collected soils during the growing season of 2010 and 2011. Specific sample dates were: 8 July, and 10 September 2010; 14 May, 04 July, and 05 September 2011. During 2010, we selected six

Table 1. Study Sites and Conditions During Summer 2011

Time since fire (years)	Location	Conditions in summer 2011
1	N 64°20.095′ W 145°41.290′	Rapidly developing herbaceous vegetation and deciduous shrubs with a charred moss layer. Almost all of the black spruce and white spruce trees killed in the 2010 fire were still standing
7	N 64°21.031′ W 145°38.301′	Groundcover dominated by regenetrating aspens and other deciduous trees, evergreen and deciduous shrubs, herbaceous perennials, and resprouting black spruce. Almost all of the black spruce and white spruce trees killed in the 2004 fire were still standing
12	N 63°55.302′ W 145°45.032′	Similar conditions to stand age 7, with greater biomass of deciduous trees. Almost all of the black spruce trees killed in the 1999 fire were still standing
24	N 63°55.481′ W 145°21.468′	Aspen stand with resprouting black spruce saplings. Near continuous understory cover of graminoids, forbs, and deciduous shrubs. Feathermoss is resprouting but not dominant. Most black spruce trees killed by the 1987 fire had fallen prior to 2011
55	N 63°55.481′ W 145°21.468′	Dense black spruce and aspen stand, with aspen mortality and thinning. Nearly 100% feathermoss groundcover. Understory is dominated by deciduous shrubs, forbs and graminoids
90	N 63°56.682′ W 145°36.162′	Dense black spruce stand with groundcover dominance split between feathermoss and lichen. Understory is dominated by deciduous shrubs, forbs and graminoids
100	N 64°20.500′ W 145°49.301′	Moderately dense black spruce and white spruce stand with nearly 100% feathermoss groundcover. Understory is dominated by deciduous shrubs, forbs and graminoids

random locations per site and compiled four soil cores per location. Random sampling locations were separated by at least 10 m. Soil cores were 10 cm deep by 5 cm in diameter. Soils collected in 2010 were used for soil biogeochemistry and fungal community analyses. We followed a similar sampling design in 2011, except that soil cores were separated by soil horizon for all analyses. We sampled the entirety of the organic horizon at each site and mineral soil was sampled to a depth of 5 cm. Organic horizon depth was variable across sites, ranging from ~5 cm at control sites to less than 1 cm at recently burned sites (Appendix B in Supplementary Material). Soils collected in 2011 were used for fungal hyphal length and enzyme analyses. Following collection, soils were placed on ice and transported to UC Irvine (Irvine, CA, USA) within 24 h of sampling. Soils were hand-homogenized and stored at either 4°C (fungal hyphal length and soil biogeochemistry) or −80°C (fungal community analyses and enzyme assays).

Soil Biogeochemistry

Soil biogeochemical measurements were made on soils collected in 2010. Moisture content was determined by drying soils to 65°C for 48 h. Soil C and N concentrations were measured by combustion on an elemental analyzer (Flash EA 1112, Thermo Scientific, Waltham, MA). Soil NH_4^+-N and NO_3^--N were extracted from a 5 g soil subsample with 50 ml 2 M KCl for 1 h. We measured NH_4^+-N concentrations using a modified Berthelot-salicylate method (Weatherburn 1967) and NO_3^--N concentrations with the vanadium method of Doane and Horwath (2003). A subsample of soil was processed to determine soil pH with a 1:2 ratio (w/v) of soil to DI H_20.

Fungal Community Structure

Soils for fungal community analysis were collected in July and September 2010. Due to restrictions in access, we were only able to sample from the 55-year burn in July 2010. Soil samples from a particular site and sampling date were pooled into a single composite sample and stored at −80°C. DNA was extracted from a 0.25 g subsample of each composite soil sample using the Powersoil DNA extraction kit (MoBio, Carlsbad, CA, USA). Three DNA extractions from each sample were pooled to obtain a better representation of the fungal community (Feinstein and others 2009) and DNA concentrations were standardized to 10 ng/μl before PCR amplification.

General fungal primers targeting the fungal 18S rRNA gene (Borneman and Hartin 2000) were modified and implemented to amplify the fungal community using a barcoded pyrosequencing procedure described previously (Rousk and others 2010; McGuire and others 2012). The forward primer consisted of the 454 Life Sciences Primer B attached to the SSU817f primer with an ''AG'' linker sequence (GCCTTGCCAGCCCGCTCAGAG TTAGCATGGAATA ATRR-AATAGGA). The reverse primer contained the 454 Life Sciences Primer A, a unique 12 base-code barcode for each PCR product, with an ''AC'' sequence linking it to the SSU1196r primer (GCCTCCCTCGCGCCATC AG-12 bp barcode ACTCTGGACCTGGTGAGTTTCC). The 12-bp bar code allowed us to pool together all of the amplicons for sequencing with sequences ultimately assigned to individual samples. Each reaction was performed in triplicate and contained: 2 μl of DNA template, 1 μl of BSA, 0.75 μl each primer, and 22.5 μl of Platinum PCR Supermix (Invitrogen, Carlsbad, CA). The reaction ran for 30 cycles of 94°C for 45 s, 52°C for 30 s, and 72°C for 90 s with a hot start at 94°C for 10 min and final extension step at 72°C for 10 min. Triplicate PCR products were combined and purified using a MoBio UltraClean-htp PCR Clean-up kit to remove primer dimers and quantitated via fluorescence with a Qubit fluorometer. Samples were then pooled in equal amounts into one sample for 454 Pyroseqeuncing and concentrated using a Purelink PCR Purification Kit (Invitrogen, Carlsbad, CA). The pooled fungal amplicons were sequenced on a Roche 454 Gene Sequencer at the Environmental Genomics Core Facility at the University of South Carolina (Columbia, SC, USA).

Following pyrosequencing, sequences were processed through the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (Caporaso and others 2010). In QIIME, sequences were quality checked, aligned, and clustered into operational taxonomic units (OTUs) at a 97% sequence similarity cutoff. One representative sequence from each OTU was chosen, and the closest taxonomic identity for each representative sequence was determined by BLAST comparison against sequences contained within the SILVA database (Pruesse and others 2007) and GenBank. Furthermore, OTUs were assigned to functional groups (for example, mycorrhizal, saprotrophic, pathogen, and others) using the BLASTn algorithm against known AFTOL sequences (Lutzoni and others 2004) in GenBank. We placed OTUs in a given function group if the representative sequence was greater than 97% similar to sequences from the AFTOL

database. OTUs that could not be reliably placed into a single functional group were listed as unknown functional groups. All sequences were deposited in the GenBank sequence read archive with the accession number SRA046762.1.

Fungal Hyphal Length

As a metric for total soil fungal abundance, we measured the length of fungal hyphae in soil. Fungal hyphal lengths were determined for each 2011 sampling date using a modified procedure from Sylvia (1992) and Brundrett and others (1994). Briefly, 5 g organic soil or 10 g mineral soil (wet weight) were extracted with a 1.5 M solution of sodium hexametaphosphate. This soil solution was passed through a 0.2-μm nylon filter, and hyphae were collected on the filter. The filter was stained with acid fuchsin, mounted on a glass slide with polyvinyl lactic acid (PVLG) slide mounting medium, and dried at 60°C overnight. We measured hyphal length at 200× using a gridline intersect method (Brundrett and others 1994) on a Nikon phase-contrast microscope (Nikon Eclipse e400, AG Heinze, Lake Forest, CA, USA). Actual numbers of samples analyzed were lower than the number of samples collected when some samples were discarded owing to difficulty viewing hyphae because of obstruction by organic matter. We measured soil bulk density at each site and in each horizon in May 2011 and used soil bulk density values to adjust results to an area basis (Appendix B in Supplementary Material).

Extracellular Enzyme Activities

We assayed the potential activities of six hydrolytic extracellular enzymes involved in C and nutrient cycling using the microplate fluorometric protocol of Saiya-Cork and others (2002) with modifications by German and others (2011). We measured the activities of: α-glucosidase (AG, a starch degrading enzyme), β-glucosidase (BG, which hydrolyzes cellobiose into glucose), β-xylosidase (BX, which degrades the xylose component of hemicellulose), cellobiohydrolase (CBH, an exocellulase), *N*-acetyl-glucosaminidase (NAG, which breaks down chitin), and acid phosphatase (AP, which mineralizes organic phosphorus into phosphate).

Potential enzyme activities were measured on organic soil samples collected from each site in July 2011. Soils were frozen at −80°C following collection and were processed for enzymatic activity within 1 month of collection. Freezing may affect the activity level of certain extracellular enzymes (DeForest 2009). However, because all samples were frozen in a similar manner, freezing likely did not alter our ability to compare activity levels across sites. In brief, 1 g organic soil (wet weight) was homogenized in 125 ml sodium acetate buffer (pH 5.0) using a hand blender. Two hundred microliters of this soil homogenate were combined with 50 μl fluorometric substrate solution in a microplate well and incubated for 1 h at 10°C. After incubation, 10 μl of 1 M NaOH was added to each well of the microplate to terminate enzyme activity. Following termination of each reaction, we used a fluorometer set at 365 nm excitation and 450 nm emission to measure fluorescence. The assay of each enzyme was replicated eight times per microplate, and each microplate included a standard curve of the product (4-methylumbelliferone, MUB), substrate controls, and homogenate controls. Enzymatic activity (nmol product released h^{-1} g^{-1} dry soil) was calculated from the MUB standard curve following German and others (2011).

Litterbag Decomposition Experiment

To assay microbial activity at each chronosequence site, we conducted a litterbag decomposition experiment. Senescent litter from black spruce (*Picea mariana* (P. Mill) B.S.P) and aspen (*Populus tremuloides* Michx.) was collected in July 2010. These are two of the dominant tree species in the region and are present at each study site. Spruce litter was collected from the 90-year control site and aspen litter was collected from the 24-year burn site. Litters were air-dried to constant mass and then placed into litterbags (10 cm × 10 cm) constructed of 1-mm fiberglass mesh and reinforced with 0.2-mm nylon. Each litterbag received 2.0 g of air-dried litter. Litterbags were deployed in September 2010 and incubated for 1 year in the field. Due to restrictions in site access, we were unable to deploy litterbags at the 55-year burn. Litterbags of each litter type were replicated five times for a total of 60 litterbags (6 sites × 2 litter types × 5 replicates). After 1 year of decomposition, litterbags were harvested and oven-dried at 60°C to constant mass. Mass loss was determined as the difference between initial dry weight and final dry weight of litter.

Statistics

To examine changes in soil biogeochemical variables and potential enzyme activity across the chronosequence, we conducted analysis of variance (ANOVA) with the time since fire as the

independent variable. Values for soil percent C, NH_4^+-N, NO_3^--N, and activity values for AG, BG, BX, CBH, NAG, and AP were log-transformed prior to ANOVA to improve normality and homogeneity of variance. We were unable to transform hyphal length data sufficiently to meet assumptions of ANOVA, so hyphal length analyses were performed on ranked data using Kruskal–Wallis nonparametric tests (Sokal and Rohlf 1995). Comparisons among means were analyzed by Tukey's HSD post-hoc contrasts. In all cases, because our chronosequence contained only a single site from each stand age, the experimental units were soil cores from within a given chronosequence site. Error terms thus represent within site variation. In addition, because we did not return to the same exact sampling locations at each sampling date, separate statistical tests were performed for each date. We used regressions to test for a relationship between litter mass loss and the time since fire and between extracellular enzyme activity and fungal hyphal length. Differences are reported as significant when $P < 0.05$. All data were analyzed using JMP statistical software (JMP v. 8.0, SAS Institute, Inc 2009).

To determine the relationship between fungal community composition and the time since fire, we employed a PerMANOVA using the Adonis function in the Vegan package of R (Oksanen and others 2012). In this model, we also included dominant vegetation type (that is, herbaceous, deciduous trees, evergreen trees), sampling date, and soil biogeochemical variables. In addition, observed number of fungal OTUs, Chao1 estimates of OTU richness, and Shannon diversity index were computed for each site and sampling date in QIIME. Chao1 values estimate actual rather than observed OTU richness of microbial communities (Hughes and others 2001). The Shannon index of diversity accounts for evenness as well as richness of each community (Magurran 1988). For fungal community analyses, the experimental unit was a single composite soil core from each chronosequence site. Diversity indices were compared statistically by averaging the July 2010 and September 2010 values and using ANOVA. We used regressions to test for a relationship between the relative abundance of fungal taxa or functional groups and the time since fire. Relative abundance data were calculated from OTU assignments to either fungal taxa or functional groups. Nonmetric multidimensional scaling plots were used to visualize similarity in fungal community composition across sites (McCune and Mefford 2006).

Results

Soil Biogeochemistry

Wildfire had a negligible short-term effect on soil biogeochemistry. In contrast, fire had significant long-term impacts on soil biogeochemical parameters (Appendix C in Supplementary Material). For example, soil moisture was significantly lower in intermediate aged sites (7, 12, and 24-year burns) but soil moisture did not differ between the control sites and the 1-year burn. Percent soil C was not affected by fire 1 and 3 months following fire, but decreased 7, 12, and 24 years post-fire. Changes in soil nutrient availability following fire were variable. Soil NO_3^- did not differ between the 1-year burn site and the control sites in either July or September 2010, but NO_3^- was significantly lower in intermediate aged sites. In September 2010, soil NH_4^+ was highest in the 1-year burn and lowest in the 6-year burn. There were no significant differences in soil pH across sites (Appendix C in Supplementary Material).

Fungal Community Analyses

Pyrosequencing yielded a total of 32,448 fungal sequences with an average of 2,496 sequences analyzed per study site for each sampling date. Rarified data showed that the total number of fungal OTUs did not differ significantly across sites ($F = 1.927$, $P = 0.222$), nor did Chao1 estimates of OTU richness ($F = 0.960$, $P = 0.519$; Table 2). However, the Shannon diversity index differed significantly across sites ($F = 10.696$, $P = 0.008$), with the 90-year control stand harboring the least diverse soil fungal community (Table 2).

Soil fungal communities varied significantly with the time since fire (PerMANOVA: $r^2 = 0.231$, $P = 0.001$; Figure 1, Appendix D in Supplementary Material). Differences in community composition across sites were primarily driven by changes in the abundance of ascomycete and basidiomycete fungi. The relative abundance of ascomycete fungi significantly decreased with the time since fire during the September 2010 sampling ($r^2 = 0.714$, $P = 0.034$), whereas basidiomycete relative abundance increased with stand age in the July ($r^2 = 0.613$, $P = 0.037$) and September 2010 samplings ($r^2 = 0.684$, $P = 0.042$; Figure 2A). The most abundant taxa at the control sites belonged to the ectomycorrhizal genus *Cortinarius* and the most abundant taxa at the 2010 burn site belonged to the ascomycete genus *Ascocryne*. Decreases in the relative abundance of basidiomycete fungi following fire were likely attributable to declines in ectomycorrhizal fungi. The relative abundance of

Table 2. Observed Number of OTUs (Operational Taxonomic Units), Chao1 Estimates of OTU Richness, and Shannon Diversity Index at 97% Sequence Similarity

Time since fire (years)	OTUs	Chao1	Shannon index
0.17	121.2 (24.2)	212.2 (46.3)	4.2 (0.1)[a]
6	142.1 (1.3)	247.2 (11.7)	4.8 (0.1)[a]
11	134.3 (14.8)	277.0 (58.0)	4.3 (0.1)[a]
23	117.6 (6.2)	226.0 (8.3)	4.2 (0.0)[a]
54	108.2	230.0	3.9[ab]
90	99.8 (1.5)	178.8 (12.6)	2.4 (0.3)[b]
100	122.3 (18.9)	256.1 (21.6)	2.8 (0.6)[ab]

The experimental unit was a single composite soil core from each chronosequence site. Data are reported as the mean of the July and September 2010 sampling dates. The error term (±1 SE) thus represents variation between these two sampling dates. Different letters indicate significant differences between sites ($P < 0.05$). The Shannon index was the only metric that differed across sites.

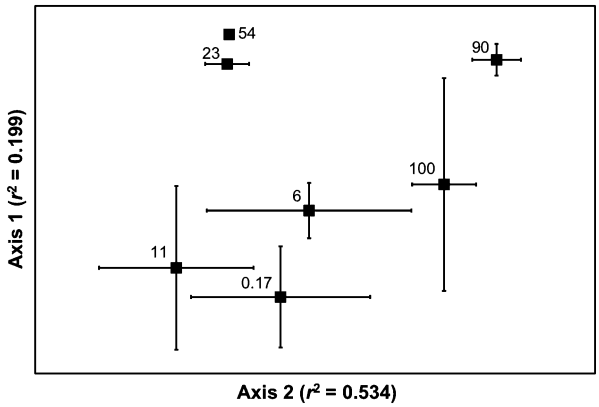

Figure 1. Nonmetric multidimensional scaling (NMS) plot showing compositional differences in fungal communities by the time since fire. Fungal communities differed significantly with the time since fire ($r^2 = 0.231$, $P = 0.001$). Experimental units and error terms are as in Table 2. Numbers adjacent to symbols indicate the time since fire (years). The final stress was 3.94 after 65 iterations.

ectomycorrhizal fungi (based on OTU assignments to functional groups) increased significantly with the time since fire in the July ($r^2 = 0.578$, $P = 0.047$) and September 2010 samplings ($r^2 = 0.739$, $P = 0.028$; Figure 2B). In contrast, the relative abundance of saprotrophic basidiomycetes did not change with stand age at either sampling date (July: $r^2 = 0.0004$, $P = 0.962$; September: $r^2 = 0.008$, $P = 0.865$; data not shown). The community composition of soil fungi was also affected by the dominant plant species and soil percent C (Appendix D in Supplementary Material). There was no overall effect of sampling date on fungal community composition (Appendix D in Supplementary Material).

Fungal Abundance

Fungal hyphal length in the organic horizon varied significantly across sites at each sampling date in 2011 ($P = 0.0001$ for each sampling date; Figure 3). In general, there were similar patterns in fungal abundance across all three sampling dates. Recently burned sites (1–12 years post-fire) had significantly lower soil fungal abundance than older sites, and hyphal lengths increased dramatically between 12 and 24 years post fire (Figure 3). Hyphal length in the organic horizon significantly decreased between the 90- and 100-year-old site in May 2011 ($P = 0.05$). There were similar but nonsignificant declines in July and September 2011.

Fungal abundance in the mineral horizon was less variable across sites. Hyphal lengths in the mineral horizon differed significantly across sites in July and September 2011 (P (July) = 0.013, P (September) = 0.0004), but the magnitude of the difference was smaller than differences in the organic horizon (Figure 3). In the mineral horizon, the 24, 55, and 90-year-old sites tended to have higher fungal abundance than the other chronosequence sites. This trend is broadly consistent with the pattern observed in the organic horizon. There was no significant difference in fungal abundance in the mineral horizon across sites in May 2011 ($P = 0.173$, Figure 3).

Extracellular Enzyme Activities

Extracellular enzyme activities varied significantly with the time since fire ($P < 0.0001$ for each enzyme, Table 3). Across all measured enzymes, fire reduced potential enzymatic activity (Table 3). The negative effect of fire on enzyme activity persisted for 7–12 years and activity values were highest in the 90-year-old control site for all measured enzymes. Carbon targeting enzymes (AG, BG, BX, CBH), N-targeting NAG, and P-targeting AP exhibited similar responses to fire. For each measured enzyme, fungal hyphal length explained a significant proportion of the variation in enzyme

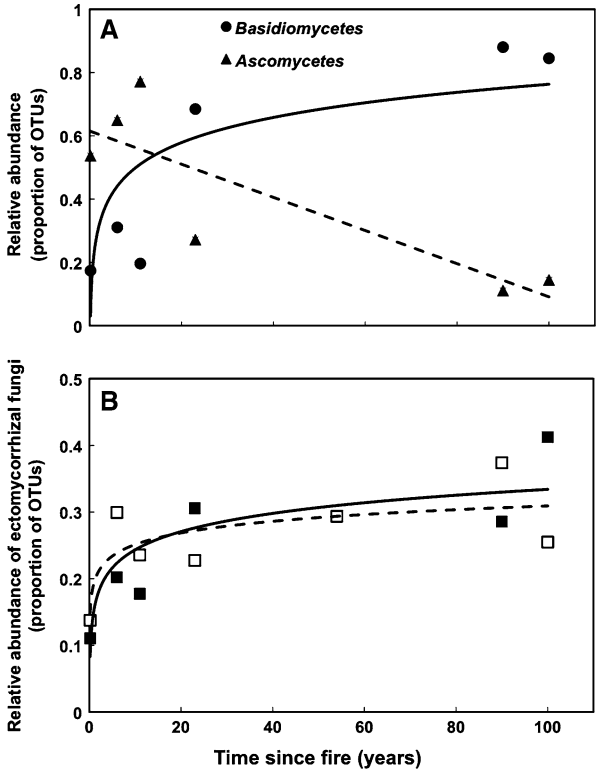

Figure 2. **A** The relative abundance of ascomycete (*triangles*) and basidiomycete (*circles*) fungi as a function of the time since fire in September 2010. *Lines* are the best-fit regressions. Basidiomycete abundance increased with stand age (*solid*, Basidio Abundance = 0.24 + 0.11 * log (time), $r^2 = 0.684$, $P = 0.042$) whereas ascomycete abundance decreased with stand age (*dashed*, Asco Abundance = 0.61 − 0.005 * time, $r^2 = 0.714$, $P = 0.034$). **B** The relative abundance of ectomycorrhizal fungi as a function of the time since fire. *Open squares* represent samples from July 2010, and *closed squares* correspond to samples collected in September 2010. *Lines* are the best-fit regressions. The relative abundance of ectomycorrhizal fungi increased significantly with the time since fire in July 2010 (*dashed*, Ecto Abundance = 0.19 + 0.03 * log (time), $r^2 = 0.578$, $P = 0.047$) and September 2010 (*solid*, Ecto Abundance = 0.15 + 0.04 * log (time), $r^2 = 0.739$, $P = 0.028$). Experimental units are as in Table 2.

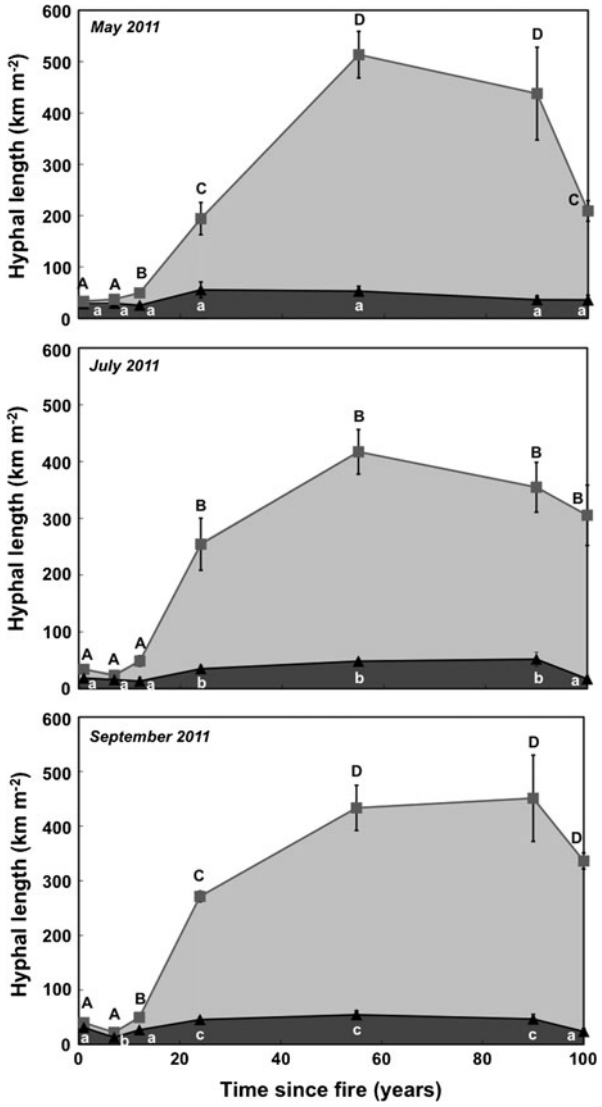

Figure 3. Fungal hyphal length in the organic (*gray squares*) and mineral (*black triangles*) soil horizons in relation to the time since fire. The experimental units were soil samples from a given site and symbols represent site mean values. Error bars (±1 SE) thus represent within site variation in hyphal length for $n = 3$–6 soil samples. *Different letters* indicate significant differences between sites at $P = 0.05$; *uppercase letters* represent organic horizons and lowercase letters represent mineral horizons. Hyphal length in the organic horizon differed across sites at each 2011 sampling date ($P = 0.0001$) and in the mineral horizon in July ($P = 0.013$) and September 2011 ($P = 0.0004$).

activity values but the relationship was logarithmic (Appendix E in Supplementary Material).

Litter Mass Loss

The percent mass loss of aspen and spruce litter increased significantly with the time since fire (Aspen: $r^2 = 0.691$, $P < 0.001$, Black spruce: $r^2 = 0.612$, $P < 0.001$; Figure 4). Decomposition constants (k) for black spruce litter ranged from 0.187 at recently burned sites to 0.360 at the 90-year control site (Appendix F in Supplementary Material). Litter decomposing in older forest stands lost 11–18% more mass than litter decomposing at recently burned sites. We observed similar mass loss patterns for both aspen and black spruce litter across sites.

Table 3. Extracellular Enzyme Activities (nmol h^{-1} g^{-1} dry soil) of Carbon Targeting Enzymes (α-Glucosidase, β-Glucosidase, β-Xylosidase, cellobiohydrolase), N-Targeting *N*-Acetyl-Glucosaminidase, and P-Targeting Acid Phosphatase

Time since fire (years)	α-Glucosidase	β-Glucosidase	β-Xylosidase	Cellobiohydrolase	*N*-Acetyl-glucosaminidase	Acid phosphatase
1	33.5 (6.5)^{ab}	604.4 (98.9)^{bc}	86.4 (11.9)^{b}	74.4 (19.6)^{bc}	955.9 (323.6)^{a}	1725.4 (387.9)^{a}
7	20.2 (3.6)^{a}	236.4 (72.8)^{a}	24.0 (7.8)^{a}	38.2 (13.4)^{a}	191.7 (40.8)^{a}	797.2 (105.8)^{a}
12	22.5 (3.0)^{a}	520.1 (77.1)^{b}	105.4 (20.3)^{b}	84.4 (24.4)^{bcd}	201.3 (23.7)^{a}	2218.4 (238.4)^{a}
24	32.0 (6.2)^{ab}	961.8 (145.5)^{bcd}	82.9 (8.7)^{b}	77.6 (19.4)^{bc}	677.6 (126.9)^{a}	1837.4 (398.1)^{a}
55	64.5 (12.1)^{bc}	969.2 (200.6)^{bcd}	103.4 (25.0)^{b}	102.9 (13.2)^{cd}	1232.8 (369.3)^{a}	5031.5 (556.4)^{b}
90	182.3 (17.1)^{d}	2017.4 (209.4)^{d}	271.7 (41.3)^{c}	277.4 (61.2)^{d}	3561.5 (627.9)^{b}	8411.2 (664.5)^{c}
100	110.5 (37.7)^{cd}	1403.3 (314.7)^{cd}	176.7 (21.3)^{bc}	190.2 (46.7)^{cd}	2704.8 (328.8)^{b}	5028.7 (904.7)^{b}

The experimental units were soil samples from a given site and values represent site means. The error term (±1 SE) thus represent within site variation in enzyme activities for n = 6 soil samples. Different letters indicate significant differences between sites at P = 0.05.

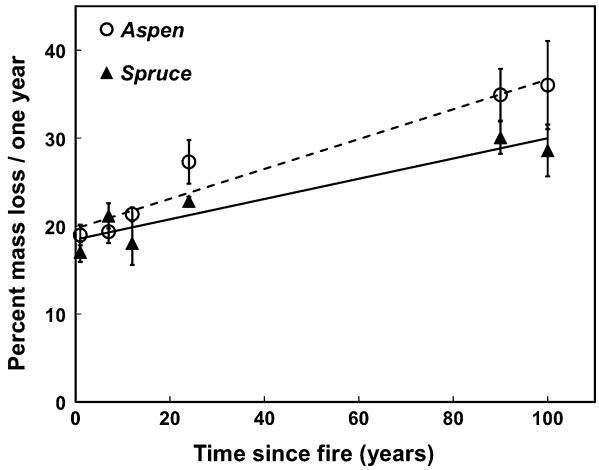

Figure 4. Percent mass loss of aspen (*open circles*) and black spruce (*black triangles*) litter after 1 year of incubation as a function of the time since fire. The experimental units were litterbags from a given site and symbols represent site mean values. *Error bars* (±1 SE) thus represent within site variation in mass loss for $n = 5$ litterbags per litter type. *Lines* are the best-fit regressions for aspen mass loss (*dashed*, mass loss = 19.52 + .17 * time, $r^2 = 0.691$, $P < 0.001$) and spruce mass loss (*solid*, mass loss = 18.50 + .12 * time, $r^2 = 0.612$, $P < 0.001$). The mass loss of aspen and spruce litter increased significantly with the time since fire.

Discussion

In this study, we characterized the response of soil fungal communities and soil C dynamics to wildfires in boreal forests. Our results indicated that boreal wildfires had short- and long-term effects on soil fungal communities, extracellular enzyme activities, and litter decomposition rates. Within 1 year following a severe wildfire, we observed a significant reduction in fungal hyphal length in the organic horizon (Figure 3). Declines in fungal abundance per unit ground area in the organic horizon likely resulted from several interacting factors. First, combustion of the uppermost layer of the organic horizon during fires resulted in a shallower organic horizon (Appendix B in Supplementary Material). In addition, post-fire reductions in fungal hyphal length may be attributable to heat-caused mortality of soil fungi. Both laboratory and field studies have indicated that fatal temperatures for microbes can be less than 100°C (DeBano and others 1998), which is well below the surface temperatures achieved during many fires (Hernandez and others 1997; Neary and others 1999). Furthermore, post-fire reductions in net primary productivity (NPP) and root exudation following

fires can decrease the input of labile C to soils, and this may in turn cause fungi to be C limited (Choromanska and DeLuca 2001). Finally, decreases in fungal biomass following fires may be mediated by post-fire changes in soil moisture, soil nutrients, and pH (Smith and others 2008; Capogna and others 2009; Bárcenas-Moreno and others 2011). Fungal hyphal length in the mineral horizon was substantially lower than in the organic horizon and did not show a strong response to fire (Figure 3). Mineral soils were likely buffered from temperature changes during the fire and post-fire changes in soil C and nutrients were primarily restricted to the organic horizon.

We initially predicted that the effect of fire on soil fungal communities would persist until aboveground plant communities recovered. Although the effect of fire on fungal communities was transient, soil fungal recovery lagged behind aboveground plant recovery. Previous work along this chronosequence found that plant biomass and productivity can recover in as little as 4 years following a fire (Mack and others 2008). In contrast, fungal hyphal length required at least 24 years to return to pre-fire levels (Figure 3). Although aboveground NPP can recover quickly, soil organic matter accumulates slowly over time as C inputs from NPP outweigh C losses from decomposition. Treseder and others (2004) found that soil organic matter at these sites increased with the time since fire and required more than 15 years to return to pre-fire levels. Taken together, these findings suggest that the recovery of soil hyphal abundance following wildfires is more strongly related to the recovery of soil organic matter than to the recovery of aboveground plant communities.

In addition to reducing fungal biomass, wildfires also altered the composition of soil fungal communities (Figures 1, 2). High-resolution studies of the entire soil fungal community following boreal fires are rare, but community fingerprinting techniques have previously documented broad changes in fungal community composition in response to fires (Bååth and others 1995; Waldrop and Harden 2008). However, these studies have been unable to indentify which fungal taxa were positively or negatively affected by fire. We found strong differences in soil fungal communities that corresponded to the time since fire. Ascomycete fungi were abundant 1 month following fire and early in forest succession. Previous surveys of aboveground fungal fruiting bodies following fires have reported post-fire fruiting of ascomycetes (Peterson 1970; Wicklow 1973, 1975; Chen and Cairney 2002; Fujimura and others 2005). Our sequence data provide additional molecular evidence for this commonly observed pattern.

In contrast, basidiomycetes were relatively scarce in the first years following fire and increased in abundance during secondary succession. We compared fungal OTUs from our study to known sequences in the AFTOL database and assigned functional groups to these OTUs if the representative sequence was greater than 97% similar to sequences from the AFTOL database. Based on functional group assignments, our data suggest that declines in basidiomycetes following fires were likely driven by decreases in the abundance of ectomycorrhizal fungi following fire and during early succession (Figure 2B). However, it is important to note that ~25% of OTUs in our study could not reliably be placed in a single functional group, either because they did not closely match an AFTOL sequence or because they matched a fungal species with an unresolved function. In particular, certain ascomycete fungi that are common following fire (for example, Pezizales) may be facultatively mycorrhizal (Dahlstrom and others 2000; Fujimura and others 2005) and could not be assigned to a single functional group. Results from previous studies lend support to our finding that ectomycorrhizal fungi decline following fires. For example, fire has previously been shown to decrease the abundance of ectomycorrhizal fungi in boreal forests (Treseder and others 2004) and to alter the composition of ectomycorrhizal fungi in pine forests (Taylor and Bruns 1999). Ectomycorrhizal fungi may decline following fires because they lack heat resistant propagules in soil or due to the lack of appropriate host plants.

Although the time since fire explained the largest proportion of the variation in fungal community composition across sites, the dominant plant species and percent soil C were also important for structuring soil fungal communities (Appendix D in Supplementary Material). These factors have previously been shown to influence soil microbial communities (Kivlin and others 2011; Barham and others 2012). However, because the dominant plant species and percent soil C are correlated with the time since fire, it is difficult to discern their individual effects on fungal community structure separately from the effect of fire.

Changes in extracellular enzyme activities across the fire chronosequence were consistent with changes in fungal abundance. For each measured enzyme, fungal hyphal length explained a significant proportion of the variation in enzyme activity (Appendix E in Supplementary Material). This finding is intuitive, because extracellular enzyme

assays reflect the total pool size of a particular enzyme in soil, and enzyme pool sizes should be related to soil microbial biomass. Our finding that enzyme activity decreases following fires is consistent with previous work (Boerner and others 2008; Artz and others 2009; Gutknecht and others 2010). Given that extracellular enzymes are the proximate agents of decomposition in soil (Burns and Dick 2002), changes in enzyme pool sizes may affect litter decay rates and soil C dynamics.

To determine how post-fire changes in soil fungal abundance and enzyme activity affect soil C dynamics, we measured litter decay rates across the chronosequence. In support of the prediction that post-fire changes in soil fungal communities influence litter decay, the decomposition rate of aspen and spruce litter significantly increased with the time since fire (Figure 4) and changes in litter decomposition mirrored patterns in fungal abundance and extracellular enzyme activity. Fire has previously been shown to decrease litter decay rates (Pietikainen and Fritze 1993; Brennan and others 2009; Williams and others 2012), but the majority of previous studies have not made a link between changes in litter decay and changes in the soil fungal community. Our finding that boreal wildfires slow microbial-mediated litter decomposition is also in agreement with ecosystem-level studies that have estimated heterotrophic respiration following wildfires and found post-fire decreases in heterotrophic respiration (Czimczik and others 2006; Amiro and others 2010). The differences in litter decay rates across sites were smaller in magnitude than the differences in fungal abundance and enzyme activity. This discrepancy is potentially due to the fact that post-fire increases in soil temperature create a more favorable abiotic environment for decomposition, which may partially offset the negative effect of post-fire reductions in microbial abundance and activity.

The results of our study should be extrapolated with caution. Because this was an observational study, we depend on the assumptions of the chronosequence approach to make inferences about time (Walker and others 2010). The chronosequence we used is not replicated in space, and thus we were unable to control for site-specific effects in our study. Although we took great care to minimize differences among sites other than the time since fire, it is possible that additional unmeasured factors may have contributed to the patterns we observed in this study. We were also unable to incorporate microbial responses to changes in burn severity or burn frequency, both of which are likely to change with climate warming and to affect soil microbial communities. Furthermore, in the present study we measured hydrolytic enzymes but did not characterize the activity of oxidative soil enzymes that control the degradation of more recalcitrant C compounds (for example, lignin). Finally, most microbial responses in this study were measured over the course of a single year, and it is possible that inter-annual variation in environmental factors may modify microbial responses to fire.

Overall, our findings indicate that fires reduce soil fungal abundance and alter fungal community composition. Soil fungal abundance required at least 24 years to return to pre-fire levels and did not recover as quickly as plant biomass. Post-fire reductions in fungal biomass corresponded to reductions in extracellular enzyme pools and slower rates of litter decomposition. These results challenge the conventional hypothesis that microbial activity increases following fires due to increases in soil temperature. Instead, our findings support the hypothesis that there is a microbial limitation to decomposition in post-fire soils (Waldrop and Harden 2008). Thus, wildfires in boreal forests may lead to decreased heterotrophic respiration 1–12 years following fires, resulting in lower soil CO_2 emissions, and constituting a negative feedback to climate warming. These data may be useful for revising coupled wildfire-C cycle models to increase their predictive power.

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